THE OCCURRENCE OF THE SPORES OF B. BOTULINUS IN BELGIUM, DENMARK, ENGLAND, THE NETHERLANDS AND SWITZERLAND. VI *

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Botulism has been recognized in Germany since 1735, but Bitter [1] wrote in 1921 that all attempts made in Europe to locate B. botulinus in nature had completely failed. In 1919 Semerau and Noack [2] expressed the opinion that the anaerobe occurred probably in regions or districts which from their geological or climatological location or incidental to contact with animal life and plant growth offered an environment suitable for multiplication and persistence. With the exception of the studies of van Ermengem [3] and Bitter, no data were available to support the hypothesis of Semerau and Noack. In view of the American findings, it suggested itself to examine a number of soil specimens collected in European countries in which human botulism had occurred. Such an undertaking was also prompted by a desire to compare the B. botulinus strains of the Old World with those of the New World. It can be stated without any fear of contradiction that nothing definite is known with regard to the various strains of B. botulinus which have been isolated in Europe. The early descriptions of van Ermengem and Leuchs [4] have with some slight modifications been copied by most of the recent writers. Moreover, the original strain of van Ermengem has been lost and fourteen so-called cultures of B. botulinus received from Germany during the last year have been found nontoxic, containing B. sporogenes or B. centro sporogenes. The only evidence which indicates that B. botulinus, type B or type A, is encountered in Germany is furnished by these observations: 1. The antitoxin prepared by the Institute of Infectious Diseases, Berlin, and supplied to us by Prof. Claus Schilling neutralizes the toxin of every American type B strain. 2. The Lister Institute of

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[1] Ergebn. d. allg. Path. u. path. Anat., 1921, 19, II, p. 762.

[2] Ztschr. f. klin. Med., 1919, 88, p. 304.

[3] Ztschr. f. Hyg. u. Infektionskrankh., 1897, 26, p. 1.

[4] Ibid., 1910, 65, p. 55.

Preventive Medicine forwarded for comparison 2 cultures of B. botulinus originally received by them from the Institute of Infectious Diseases, Berlin. One culture (No. 95) was nontoxic, while the other (No. 94) furnished a pure growth of B. botulinus, type A, which is identical with the 70 American type A cultures. It has been impossible to determine whether this culture is a descendant of the original one isolated by Gaffky [5] from the white beans responsible for the Darmstadt outbreak. In 1910 it had been demonstrated by Leuchs [4] that the strain isolated by Gaffky differed immunologically from van Ermengem's original culture. On a comparative basis it appears not unlikely that Gaffky's culture was identical with what is known today as B. botulinus, type A, and if so is probably the only type A culture which has been isolated in Europe. It was expected that the examination of soil specimens from Europe might possibly confirm or enhance this interpretation.

Besides Germany, a number of European countries have reported outbreaks of botulism. For example, van Ermengem [6] described 2 outbreaks which occurred in Belgium, 1 at Ellezelles in Hainau, and 1 at Jseghem in West Flanders. The observations reported by Madsen [7] and Hoeg [8] dealt with intoxications which followed the consumption of spoiled fish in Denmark. One outbreak each has been reported from the Netherlands and Switzerland. Unfortunately Blattmann,[9] who described cases of botulism due to smoked ham in Switzerland, failed to mention the exact location of the outbreak. One botulism observation was made in England [10] in 1860, and one in France [11] in 1875, in which it was stated that the poisonous sausages had been prepared in England. However, according to Savage [12] "botulism is unknown, or at least unrecognized," in England. This statement, made by the best known English authority on food poisoning and food infections, prompted the desire to examine soil specimens from England. On account of the supposed absence of botulism it was suspected that the soil of this country contained either none or, at most, very few spores of B. botulinus. Furthermore, by analogy with conditions in the United States it was reasoned that the heavily populated areas of the British Isles and

[5] Lafar's Handbuch d. technisch. Mykologie, 1905-08, 2, p. 453.
[6] Handb. d. pathog. Mikroorganis., 1912, 4, p. 918.
[7] Centralbl. f. Bakteriol., I, Ref., 1905, 37, p. 373.
[8] Hospitalstid., 1915, 58, p. 300.
[9] Cor.-Bl. f. Schweiz. Aerzte, 1909, 39, p. 18.
[10] Brit. and For. Med., Chir. Rev., 1860, 25, p. 142.
[11] Ann. hyg. pub., 1875, 43, p. 472.
[12] Food Poisoning and Food Infections, Cambridge, 1920, p. 8.

of Europe would harbor few spores on account of the century-old manuring and tilling of the soil, and if present in such an environment that only type B would probably be encountered. That this assumption is correct is apparently proved by the findings reported in the next paragraph.

EXPERIMENTAL DATA

The technic employed for the study of the European soil specimens was identical with that reported in papers I, II, III, and IV of this series. This bacterio-geographical study would have been impossible without the hearty cooperation of several colleagues and friends. It is a great pleasure to acknowledge and to express again the sincerest thanks of the Hooper Foundation for the kind assistance rendered.

The observations made on 165 specimens are herewith briefly reported.

Belgium.—Through the assistance of Dr. Paul Fabry, exchange student at the University of California, 3 samples of soils were procured from Prof. E. Malvoz, at Liège, Belgium. Two cultures contained B. botulinus, type B.

Denmark.—Dr. H. O. Schmit-Jensen of Prof. C. O. Jensen's laboratory in Copenhagen, collected and forwarded in collaboration with the Lime Control Department of the Statens Planteavls Laboratorium, in June, 1921, 54 samples of soils, compost, etc., from Copenhagen and different parts of Denmark. The origin and character of the specimens are briefly as follows:

Eight garden soils [serum laboratory, botanical garden (2), Söborg, Gjentofte, Endrup, Tröröd, Copenhagen].

Twelve field soils [serum laboratory, experiment field, celery field, night soil, cucumber field, cabbage field (2), Söborg, Gjentofte, Emdrup, Tröröd bean field and leek field; all near Ullerup, Amager].

Four sewage discharging berths.

Five composts, 1 roots and leaves, etc.

Twenty-five soils from Nordby; Sorvad; Risby, Snesere; Haslund, pr. Randers; Sønderby; Borre, Moen; Saxild, Odder; Ll. Karleby, Roskilde; Hornskov, Sjørslev; Riisgaard, Grønbaek; Hauerbaek, Kjellerup; Ullerup, pr.Hurup; Baech, pr. Vonsbaech; Sommersted; Fodby, Fodby; Simonsgaard, Rutsker; Spagergaard, Nylars; Nøore, pr. Rødby; Alsonderup, pr. Hillerød; Heldumboel, Lemvig; Aistrup, pr. Sulsted; Gravlev, Støvring; Birkely, pr. Ulsted; Arvelund, Nakskov; Hammerhus, Tørring; Aale.

The following cultures proved toxic on repeated examination:

Compost (3 yrs. old) Horticulture "Nykastrupgaard, Amager, B. botulinus, type B.

Soil from Aistrup, pr. Sulsted, B. botulinus, type B.

Soil from Birkely, pr. Ulsted, B. botulinus, type B.

A sample of remnants of roots and leaves in Kongelunden, Amager, near Copenhagen, soil from Hammerhus, Tørring, from Sorvad and from Hornskov, Sjørslev, produced weak toxins.

Summary: 8 garden soils, none; 37 field soils, 2 type B, 3 weak toxins; 4 sewage discharging fields, none; 5 compost, roots and leaves, etc., 1 type B, 1 weak toxin.

Total: 54 samples with 3 (6) or 5.5 (11.1) %, positive cultures.

England.—Dr. William G. Savage, County Medical Officer of Health. Somerset County Council, Weston-super-Mare, kindly organized the collection and shipment of 64 samples of soil from the following localities in England: Colchester, Rhondda, Durham City, Wolingham, Shincliffe, Shotley Bridge;

Penycae Ruabon, Eastbourne; Ealing; Gloucester; Brighton; Shrewsbury; Timperley (Cheshire); Derbyshire; Isle of Wright; Breinton (Hereford); Weston-super-Mare.

The following samples furnished toxic cultures:

Surface soil near wood well enclosed by trees, at foot of the South Downs, near Eastbourne—B. botulinus, type B.

Soil from a heavy manured garden which had had road sweepings and horse manure dressings each year; 1 ft. deep in Ealing—B. botulinus, type B (surface soil sample of the same place, negative).

Surface soil on hillside, manured 1920, growing oats, South Downs, Brighton —B. botulinus, type B.

Soil 1 ft. deep in garden (kitchen), manured regularly with cow dung and horse manure, Shottle Hall, Derbyshire—B. botulinus, type B.

Surface soil from garden used for growing vegetables, heavily manured with farm manure each year and produces prolific crops, Breinton, near Hereford—B. botulinus, type B.

Surface soil from a field growing wheat at Shincliffe, and surface soil from sewage farm at Shotley Bridge, near Durham, produced weakly toxic cultures.

Summary: 47 garden and orchard soils (mostly manured), 4 type B, 2 weak toxins; 7 field soil, 1 type B, 1 weak toxin; 7 virgin soils, negative; 3 sewage farms, 1 weak toxin.

Total: 64 samples with 5 (9) or 7.8 (13.1) %, positive cultures.

The Netherlands.—Prof. D. A. de Jong, University of Leiden, Holland, furnished in March, 1922, 10 samples of soil which had been collected in Zeeland, Zuidland, Gelderland, Groningen, Utrecht, Drenthe, Friesland, Noord Holland, Noord Brabant and Limburg.

One specimen from Gelderland and one from Drenthe furnished B. botulinus, type B. The cultures prepared with the samples from Zeeland, Zuidland, Friesland and Noord Brabant were toxic. The filtrates produced typical botulism symptoms on feeding to guinea-pigs and mice, but neither type A nor type B nor a polyvalent antitoxin protected the animals against the intoxication.

Summary: 10 samples of soil, field or garden, 2 type B, or 20% positive cultures.

Switzerland.—Through the assistance of Prof. Dr. R. Burri, Schweizerische Milchwirtschaftliche und Bakteriologische Anstalt, Bern-Liebefeld, the laboratory received:

Sixteen samples of soil and dirt (meadows treated with liquid manure, 4; treated with cow's manure, 4; garden soil treated with cow's manure, 4; street dust, 2; and virgin soil from Alpine meadows of Canton Tessin and Bern, 2).

Eighteen specimens of vegetables (white cabbage; savoy, 2; white turnip, 2; dwarf peas; cauliflower, 2; white orach, preserved peas, yellow turnip, red beets, potato roots, silver beets, salad beets, Brussels sprouts; salad treated with liquid manure, 2).

The following specimens furnished toxic cultures:

Surface soil from meadow treated with liquid cow's manure, 1 B. botulinus, type B; 2 surface soil from cultivated field treated with cow's manure, 1 B. botulinus, type B, 1 weak toxin; 3 garden soil treated with cow's manure, 1 B. botulinus, type B; 1 salad from Köniz, treated with liquid manure, 1 B. botulinus, type B; 1 cauliflower, 1 B. botulinus, type B; 1 silver beets (Silber Mangold), 1 B. botulinus, type B; 1 red beets and 1 potato roots, 2 weak toxins; 1 Brussels sprouts, B. tetani.

Summary: 4 meadow soils treated with cow's manure, 1 type B; 4 cultivated fields treated with cow's manure, 1 type B, 1 weak toxin; 4 garden soil treated with cow's manure, 3 type B; 2 Alpine meadow soils, negative; 2 street dust, negative; 18 vegetable specimens from manured gardens, etc., 3 type B, 2 weak toxins.

Total: 34 samples with 8 (11) or 23.5 (35.2) %, positive cultures.

The essential results detailed in the preceding paragraphs are briefly sumrized in table 26.

TABLE 1

RESULTS OF EXPERIMENTS WITH SOILS FROM VARIOUS EUROPEAN COUNTRIES

Country and Locality	Specimens	Total Number of Specimens Examined	Number of Toxic Cultures	Total Number of Typed Cultures	Type B	Untyped	Percentage of Total Toxic Cultures	Percentage of Typed Cultures
Belgium:								
Unknown	Soils	3	2	2	2	—	—	—
Denmark:								
Copenhagen-Amager	Garden soil (8); field soil (12); sewage berth (4); compost (5)	29	2	1	1	1	6 8	3.4 } 5.5
Different parts of the country	Field soils	25	4	2	2	2	16.0	8.0 }
England:								
Different parts	Garden and orchard soil (47); field soil (7); virgin soil (7) and sewage farms (3)	64	9	5	5	4	13.1	7.8
Netherlands:								
Different parts	Meadow soil	10	6	2	2	4	(60.0)	(20.0)
Switzerland:								
Vicinity of Bern and one Canton Tessin	Soils (16) and vegetables (18)	34	11	8	8	3	35.2	23.5
Total		165	34	20	20	14	20.6	12.7

A total of 165 soil and vegetable specimens have been examined. Thirty-four, or 20.6%, of the samples furnished toxic cultures, but only 20, or 12.7%, of them have been identified as toxins of B. botulinus, type B. It must be stated at once that the cultures prepared with the European specimens exhibited one striking peculiarity, namely, the toxins generated in peptic digest-liver broth and injected into guinea-pigs in 2 c c amounts failed to kill in less than 48 hours. In fact, most of the toxins were not fatal until 3 to 4 days had passed. Previous experience had taught that these weakly toxic cultures were the probable result of the presence of relatively few heat-resistant spores. This interpretation was further supported by the observation, repeatedly made, that the heating of these soil suspensions at the temperature of the Arnold sterilizer for more than 15 minutes invariably produced nontoxic enrichment cultures. In the experiments described in previous

papers this had been shown to be the case only when the original number of spores in the soil was a small one. Isolation and purification of B. botulinus from European soil samples were therefore found exceedingly difficult. Only one strain has thus far been enriched sufficiently to insure an isolation of a pure culture.

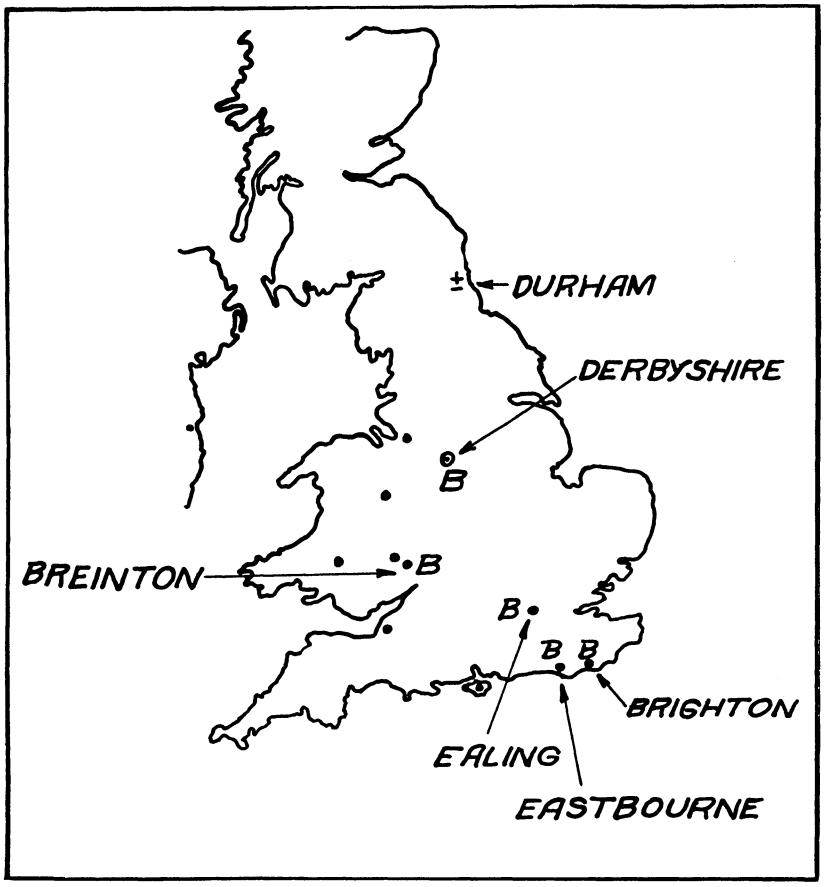

Chart 1.—Distribution of B. botulinus in England

The data secured with European field specimens confirm in a surprising manner the findings made in the United States, namely, that when type B is isolated the number of spores in the soil and its products is relatively small. Extensive cultivation suppresses B. botulinus and apparently eliminates type A. Every statement or conclusion which

has been made in the discussion dealing with the telluric distribution of B. botulinus in America can, therefore, in a general way, be applied to the conditions in Europe.

Only 3 of the 54 Danish samples contained B. botulinus, type B. This survey is sufficiently extensive to permit the conclusion that B. botulinus is rarely encountered, and then only in field soil or in composts. Garden or field soil heavily polluted with sewage gave nontoxic cultures.

From a geographical standpoint, B. botulinus is widely distributed in England, as indicated by chart 1. Considering, however, the fairly large collection of specimens studied, it must be concluded that the number of organisms is relatively small, because of 64 samples only 4 garden soils and 1 field soil contained B. botulinus, type B. One specimen from Breinton produced a potent toxin, and the organism was enriched in a fair state of purity. Cultures made from virgin soil samples were free from B. botulinus and allied anaerobes, as, for example, B. sporogenes, Vibrion septique, etc. Attention is again called to the absence of B. botulinus in the soil from sewage farms.

Six, or 60%, of the 10 soil specimens obtained from The Netherlands produced toxic cultures. Two cultures contained B. botulinus, type B, toxins. The purification of one strain is expected in the very near future. Four cultures killed guinea-pigs with characteristic symptoms on feeding (2 c c) in less than 20 hours, but neither type A nor type B, nor a polyvalent antitoxin protected the animals against this toxin. The repeated demonstration of a botulinus-like toxin in anaerobic enrichment cultures prepared with heated soil specimens suggests two questions:

1. Does the soil of The Netherlands harbor an anaerobe similar to that isolated by Bengtson [13] in the United States from the larvae of Lucilia Caesar?

2. Are all clinical cases of botulism in Europe really caused by the poison of the same toxicogenic anaerobe which has been repeatedly isolated and studied in America?

These questions can be solved only when a specific antitoxin against the anaerobe of Bengtson is available. Insurmountable difficulties have been encountered in the numerous attempts to isolated this toxin-producing anaerobe from the enrichment cultures of the Dutch soil samples. It is, of course, known that the colonies of the anaerobe of Bengtson in mixed deep agar cultures resemble closely those of other

[13] Public Health Rept., 1922, 37, p. 164.

nontoxic anaerobes or facultative aerobes. Several hundred colonies have been picked without meeting a toxic strain. These studies are being continued. Apropos of the question of whether or not all clinical cases of B. botulism in Europe are caused by the same anaerobe as that isolated in America, the following report of Bitter [14] is of interest. He isolated in 1918 and 1919, from a pickled herring and a spoiled ham, 2 strains of an anaerobe, which he identified on toxicological, morphologic and biologic grounds as those of B. botulinus. An attempt to classify these strains by means of antitoxin furnished by the Institute of Infectious Diseases, Berlin, was not successful. In the opinion of Bitter this failure must be attributed in all probability to the deterioration of the serums. He did not note the least protective action against the toxins of his strains. In the light of the observations made in this laboratory this statement opens up a new and important aspect. One of the antitoxins used by Bitter is identical with that used by us, and it has been furthermore established that this antitoxin neutralizes the toxin of all the American type B strains. The failures of Bitter may, therefore, be the result of two conditions: (a) the serums used by him were really deficient in antitoxin, or (b) the cultures contained neither a type A nor a type B toxin but an immunologically new poison. Whether this toxin is that of the Bengtson's anaerobe or possibly a B. botulinus, type C, cannot be determined from the description of his bacteriologic analysis. It is stated that his strains are highly proteolytic. The anaerobe isolated from Lucilia Caesar lacks this property, and it is not unlikely that Bitter's cultures were impure. The observations made previously on four untypable toxic cultures of Dutch soil prompt these hypotheses. They deserve the earliest attention of investigators interested in the etiology of the clinical picture generally designated as botulism.

An analysis of the cultural results made on Swiss soil and vegetable specimens reveals a high percentage of positive cultures. Eleven, or 35.2% of the 34 samples produced toxic cultures, and 8, or 23.5%, contained B. botulinus, type B. Soil or vegetables derived from manured meadows, cultivated fields or gardens furnished the spore-containing samples. Two virgin soil specimens gave nontoxic cultures. This number is obviously inadequate to venture a comparison with the conditions observed in the United States. It is not unlikely that the soil of the Alps may harbor B. botulinus quite frequently. The factors which govern the distribution of B. botulinus in California are probably

[14] Ref. 1, p. 791.

duplicated in Switzerland and may explain the high percentage of positive samples collected. Experiments are contemplated to verify these statements in the very near future.

The cultural analysis of soil and vegetable specimens obtained from Belgium, England, The Netherlands and Switzerland has definitely shown that B. botulinus, type B, exists in the Old World. This anaerobe has been found in countries in which human botulism is rare or unrecognized. As a rule, the spores are decidedly less numerous in a European than in an American soil specimen. Unfortunately, the process of purification of 2 strains has not advanced sufficiently to permit the testing of the heat resistance of the spores. However, a number of observations made incidental to the isolation of this anaerobe from toxic enrichment cultures have indicated that the spores fail to survive temperatures of 100 C. for more than from 10 to 15 minutes. Exposure of from 25 to 50 gm. of European soil containing the spores of B. botulinus to the temperature of boiling water of from 20 to 30 minutes produced nontoxic enrichment cultures. On the other hand, the resistance of these soil spores to temperatures below 100 C. is probably greater than is generally assumed from the studies of van Ermengem,[6] who found that the spores of his strains withstood a temperature of 85 degrees for less than 30 minutes. Several soil specimens from Belgium, England, The Netherlands and Switzerland have been heated for 2 hours at 85 C., and repeated tests have furnished toxic enrichment cultures.

The heat resistance of the spores of B. botulinus has apparently little or no connection with the occurrence of human botulism in Europe. According to Bitter, only 2 outbreaks are known to have followed the consumption of canned foods—one due to home-canned string beans and one due to commercially packed burbot. About 68 of the known outbreaks in Belgium, Denmark, Germany and Switzerland have been caused by food which had not been subjected to severe processing temperatures. It is also pointed out that the prevalence of botulism in country districts is frequently due to inadequate preservation incidental to the careless and insanitary treatment to which the raw material is subjected by the rural population. Home slaughtering and preservation of pork products in the form of sausages is so widely practiced in Europe that it is not at all surprising to find that about one-half of the botulism outbreaks are caused by this type of food. Accidental contamination of these products with infected soil occurs only under the most insanitary conditions of rural districts. It must

therefore be expected that human botulism due to meat, fish or animal products occurs mainly in the regions in which the B. botulinus soil index is relatively high.[15]

CONCLUSIONS

B. botulinus, type B, has been demonstrated in soil and vegetable specimens collected in Belgium, Denmark, England, The Netherlands and Switzerland.

B. botulinus, type A, has been found consistently absent.

The spores are widely distributed, but they are neither numerous nor very resistant to heat.

[15] Since this paper has been written, one of the authors (K. F. M.) has investigated an outbreak of botulism due to commercially packed potted meat at Loch Maree, Scotland. The wild duck paste was understerilized, exhibited no signs of spoilage and contained, according to the careful studies of Mr. Bruce White of Bristol University, B. botulinus, type A., and its toxin.

Dr. J. Williamson Tocher, Aberdeen, Scotland, has isolated B. botulinus, type A., from the spleen of a horse, which suffered from "grass sickness." Dr. J. B. Buxton of the Wellcome Research Laboratory has also demonstrated the toxin of B. botulinus, type A., in the blood serum of horses which have recovered from the same disease.

During the last 3 years, 2 outbreaks of botulism due to smoked ham have occurred in Switzerland. The toxin has been demonstrated but not typed in one instance.